Universität Basel

Research Project

Revisiting singular var gene choice in malaria parasites using CRISPR-Cas9 technology

Third-party funded project

Project title Revisiting singular var gene choice in malaria parasites using CRISPR-Cas9 technology
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The malaria parasite Plasmodium falciparum proliferates in the blood stream through consecutive rounds of red blood cell (RBC) invasion and intracellular multiplication. During each replication cycle some parasites cease to multiply and differentiate into gametocytes, which are essential for malaria transmission via the mosquito vector. Antigenic variation allows the parasite to escape adaptive immune responses during blood infection and hence to establish chronic infection and secure onward transmission to other human hosts. The major clonally variant antigen is known as P. falciparum erythrocyte membrane protein 1 (PfEMP1) and is exposed on the surface of infected RBCs (iRBCs). In addition to facilitating immune evasion, PfEMP1 also mediates the sequestration of iRBCs in deep tissue, which contributes strongly to the development of severe pathology. PfEMP1 is encoded by the var gene family comprising 60 genes, which are all located in transcriptionally silenced heterochromatic regions of the genome. One single var gene escapes silencing and becomes activated to express the corresponding PfEMP1 variant. Antigenic variation of PfEMP1 is brought about through in situ switches in the mutually exclusive activation of var genes. Despite extensive research in this field the regulatory mechanisms securing the activation of only a single var gene are still largely unknown. However, several lines of evidence indicate that this process may take place in a unique dedicated var gene expression site (VES) at the nuclear periphery. Here, I propose to use CRIPSR-Cas9-based approaches to test this model and to identify and characterise the factors responsible for singular var gene choice.One major part of the research proposed here will focus on the functional analysis of the histone methyltransferase PfSET10. PfSET10 is an epigenetic regulator known to be involved in var gene activation and the only protein known so far to localise exclusively to the proposed VES structure. We will employ conditional expression systems to either induce or deplete the expression of tagged PfSET10. Fluorescence microscopy and transcriptional profiling experiments will be used to assess if PfSET10 is sufficient and/or required for var gene activation. We will use ChIP-seq experiments to test if PfSET10 associates exclusively with the active var locus or if it also targets other virulence genes for activation in the VES. We will use PfSET10 as a bait to identify and characterise additional components of the VES by protein mass spectrometry followed by functional analyses. To complement these experiments, we will use CasID, a recently established method for the in vivo biotinylation of chromatin-associated proteins at specific genomic sites, as an unbiased approach to identify factors associated with the active var gene promoter. We will also attempt gaining insight into the mechanisms controlling mutually exclusive var gene expression by comparing var gene transcription and PfSET10 expression between asexual parasites and gametocytes, the latter of which do not express PfEMP1. The results anticipated from this comprehensive and purposeful set of approaches will

deliver unprecedented insight into the mechanisms that regulate mutually exclusive var gene activation and thereby facilitate expression and antigenic variation of the major virulence factor of P. falciparum malaria parasites.

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